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QUANTITATIVE ESTIMATION OF PROTEIN IN URINE*

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In normal conditions of health there is no appreciable amount of protein in urine. Very minute quantities of protein may be demonstrated, however, if special delicate methods are employed, but they are of no clinical importance. In Bright's disease and other conditions, albumin, or more properly speaking, protein is found in varying amounts in the urine.

Proteinuria is an old observation. In 1673, Frederik Dekkers,[1] a professor of medicine at Leyden, wrote:

> "The urines in phthisics . . . . are limpid and clear, especially if not boiled. Indeed I have observed these placed over a flame too soon become milky, . . . if a drop or so of acetic acid be added and it is exposed to the cold air, soon a white coagulum falls to the bottom."

Subsequently, albuminuria was more fully described by Domenico Cotugno[2] of Naples, in 1775. Doctor William C. Wells, a native of

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Charleston, South Carolina, educated at Edinburgh and then a physician to St. Thomas Hospital, London, drew attention to the frequent association of albuminuria and dropsy after scarlet fever. His observations[3] are noteworthy because they were the first to call attention to a quantitative measure of protein. He said:

> "In about a third of the cases in which serum was detected in the urine, its quantity was small, the bulk of the coagulum produced by heat and nitrous acid, . . . being from one-tenth to one-fortieth of that of the urine."

Two years later, John Blackall[4] of Exeter described both the acute and chronic forms of glomerulonephritis that followed scarlatina. In writing about the protein of urine and dropsy he remarked:

> "In addition there is another infinitely more important, its property of coagulation by heat."

It remained for Richard Bright to describe the true association of dropsy and albuminuria. His monumental book,[5] "Report of Medical Cases," published in 1827, gave complete details of twenty-four patients with dropsy, albuminous urine and kidney disease. Eighteen cases with complete anatomic findings were also included in this series. The clarity of his descriptions and the brilliance of his interpretations at once focused attention on the subject of kidney disease. So complete and accurate were his studies that they have remained for a hundred years a model of perfection and the disease itself is named after its delineator.

How does this protein get in the urine? In health blood flows to the kidneys, through a series of arteries of diminishing size until it breaks down into an arterial net-work or plexus called the glomerulus. All along this plexus a thin single cell membrane separates the blood from the inside of a large cup or funnel-shaped structure called Bowman's capsule. Free passage of water and salts, as: chloride, urea, glucose, and so forth, takes place from within the capillary tuft to Bowman's capsule. In fact, all substances except serum proteins freely flow through the membrane to begin the formation of urine. Serum proteins are such valuable substances that the body guards them zealously, preventing their escape in urine.

Bright's disease and other conditions affect this glomerular membrane. No longer is it able to retain these precious circulating proteins; but they are able to pass out in urine. Depending upon the diseased state, a variable amount of serum protein leaks through the kidneys.

Continued loss of this important body material is followed by serious consequences. With every heart beat, pressure is exerted on the blood in the finer capillaries tending to drive its more fluid components into the tissues. This drive is restrained by an opposite force called osmotic pressure. Serum proteins constitute the major portion of this sucking back action or colloidal osmotic pressure. There are a number of circulating proteins as: serum albumin, serum globulin, euglobulin, pseudoeuglobulin, hemoglobulin and so forth. Of these serum albumin is most important for one gram exerts an osmotic pressure of 79.5 mm. H_2O, while one gram of serum globulin has only 19.5 mm. H_2O pressure. At all times, there must be sufficient osmotic pressure to overcome capillary blood pressure thus preventing the escape of fluid into these tissues or edema. A continued loss of proteins through the urine will result in a diminution of serum proteins. There are certain critical levels of serum proteins. When the serum proteins fall below 3.4 grams per cent or the serum albumin below 2.0 grams per cent edema is imminent.[6]

Early in the disease the body attempts to avert this disaster by manufacturing more plasma protein. As long as the body can make enough protein to replace that lost in the urine no symptoms will arise. Under certain conditions these large demands cannot be met. The situation then is like a man who married a spendthrift wife, for he is unable to earn enough to support her capricious expenditures. Disaster is in the offing.

At that stage the well known signs and symptoms of the nephrotic syndrome appear. Massive edema, large amounts of protein and doubly refractile bodies appear in the urine; hypercholesteremia and a low basal metabolic rate are the most obvious findings. There are no changes in the cardiovascular system as evidenced by the normal blood pressure, the normal heart size and the normal appearance of the retinal vessels. Neither is there retention of nitrogen products in the blood stream, hematuria, nor functional alterations

of the kidneys as judged by the normal concentrating ability of the kidneys, the normal phenolsulphonephthalein and the normal urea clearance. These findings all indicate that the nephrotic syndrome is a basic degenerative process.

Edema of the nephrotic syndrome is the result of the low plasma protein content of the blood stream. It has been proven experimentally.[7] If dogs are bled daily and their red blood cells washed repeatedly with saline to free them from serum plasma and then re-injected, edema will develop. By this procedure,[8] called plasmapheresis, the dog's plasma proteins are reduced by repeated bleedings, without reducing the red cell count. Edema can be induced practically at will by reducing the osmotic pressure below critical levels.

Serum proteins may be reduced by other means. Normally, an adequate intake of protein[9] is necessary for the manufacture of serum proteins by the body. Insufficient protein intake means that many essential amino acids needed for plasma synthesis are lacking. Starvation results in the so-called hunger edema. Daily wear and tear of protein depletes the existing stores of serum proteins, very little new protein is being manufactured so edema appears. Certain diseases, as cirrhosis of the liver[10] or primary carcinoma of the liver, that invade the alleged sites of serum production result in edema, presumably by interference with serum protein synthesis.

Proteinuria is an important laboratory finding. Different forms of Bright's disease affected the kidneys by the excretion of variable amounts of protein and sediment in the urine. Red blood cells, white blood cells, hyaline, granular and fatty casts, cylindroids, crystals, and so forth are easily recognized sedimentary changes. Also there is a method for making a quantitative estimation of the formed elements, the Addis count.[11] The presence of protein in urine is easily detected by heat, acetic acid or sulphosalicylic acid. All of these findings are most helpful in establishing the forms of Bright's disease.

A quantitative expression of the loss of protein gives valuable information. Urinary protein has been shown to be serum protein.[12] An accurate daily record of these losses is of great help in the man-

agement of these renal disorders. The analogy to the treatment of diabetes mellitus is obvious. Not only must presence of glycosuria be known in the proper handling of the diabetic, but the quantity of glucose spilled is also important.

There is at present no entirely satisfactory method for the quantitative estimation of protein in urine by the average clinical laboratory. This led to a review of the different methods suggested in the past with a view of devising a simple accurate method.

Some Present Methods of Protein Determination

A perusal of the methods for the quantitative determination of protein in urine revealed five widely accepted methods. They included: Esbach,[13] Kingsbury-Clark,[14] Exton,[15] Shevky-Stafford,[16] and Van Slyke.[17] Fifty-five specimens of urine containing variable amounts of protein were tested simultaneously by each of the five methods. The protein content as determined by the first four of these tests was compared to the values obtained by the Van Slyke method, a generally accepted standard procedure.

Each of these methods is based on a different principle.

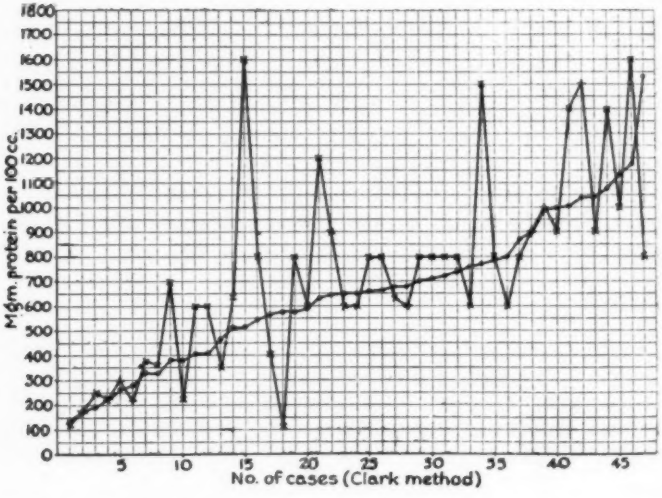
The Esbach test relies on the amount of protein precipitated by a mixture of picric and citric acids. Twenty-four hours are needed to allow the precipitate to accumulate and settle at the bottom of the tube. The quantity of protein in grams per liter of fluid is the amount of precipitate at the bottom of the special tube read directly from a semi-logarithmic scale etched on the tube. In other words, the Esbach test is a gravitational precipitation method of protein estimation.

The Kingsbury-Clark method is done in the following manner: Clear urine is added to the 2.5 cc. mark of a special test tube and 3% sulphosalicylic acid added to the 10 cc. mark. The mixture is stoppered, mixed and allowed to stand for ten minutes. At the end of that time the turbidity of the specimen is compared to a set of known standards and readings are expressed as milligrams of protein per 100 cc. of fluid. Standards used in this test represent 10, 20, 30, 40, 50, and 100 milligrams of protein per 100 cc. of urine.

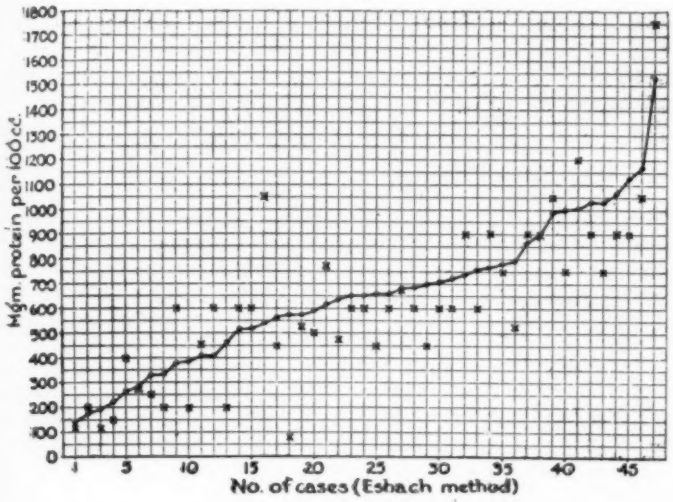
Exton's method is really a slight modification of the Kingsbury-

Clark test. The composition of this reagent is 50 gm. of sulphosalicylic acid and 10 gm. of sodium sulphate dissolved in 800 cc. of distilled water to which 25 cc. of 0.4% aqueous solution of bromphenol is added and then the whole solution is made up to one liter volume by the addition of distilled water. Equal quantities of clear urine and reagent are mixed and allowed to stand ten minutes before comparison is made with standards. Exton suggested 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mgm. protein standards per 100 cc. of urine. The small amount of bromphenol in the reagent is said to impart a yellowish tinge and make comparison easier.

The Shevky-Stafford method is an improvement of the older Tsuchiya's procedure. It is a centrifuge precipitation method. Eight cc. of urine and 5 cc. of an alcoholic solution of phosphotungstic acid are added to a centrifuge tube, whole last cubic center is drawn out like a pipette. The mixture is stoppered, inverted three times



Graph 1—Comparison of Protein Determinations by the Kingsbury-Clark and Van Slyke methods. The solid line connecting the dots represents the values found by the Van Slyke method, the line connecting the crosses the Kingsbury-Clark method.



Graph 2—Comparison of Protein Determinations by the Esbach and the Van Slyke methods. The solid line represents the Van Slyke values, the crosses the Esbach values.

and allowed to stand for exactly one minute. Then it is centrifuged at 1800 revolutions per minute for fifteen minutes. The amount of precipitate in cc. is read directly from the scale on the tube and multiplied by a factor to convert it into milligrams of protein per 100 cc. of urine.

Van Slyke's manometric method is well known. Total nitrogen of urine is determined, then the nonprotein nitrogen. By subtracting the latter from the former, protein nitrogen is found, which, when multiplied by 624, yields the total protein content.

Comparative Tests.

Two of the methods employed showed a fairly good correlation to the Van Slyke manometric method. Small quantities of protein, less than 100 mgm. per 100 cc. or 1 gm. per liter are reliably detected by the Kingsbury-Clark method. These results are best

shown in Graph No. 1. When larger amounts of protein are present the Esbach method appeared to give good agreement with the Van Slyke results, although consistently below the latter. A study of these results showed a factor of 1.54 was necessary. All Esbach readings were multiplied by 1.54 and there was a reasonably close correlation between it and the Van Slyke readings (Graph No. 2).

Neither the Shevky-Stafford nor the Exton method gave consistent results. It was not possible to correlate these readings with the Van Slyke values or the values obtained by the other methods. Previous failure and inability to obtain duplicate checks raised the question of the principles involved in these tests.

Factors Concerned in the Precipitation of Protein.

Three important factors are concerned with the precipitation of protein: the nature of the protein, the menstruum or solution in which the protein is dissolved, and lastly, the precipitant added.

It has been repeatedly shown that the protein excreted in the urine of patients is serum albumin, serum globulin, and pseudo-globulin. Their isoelectric points are pH 4.7, 5.5 and 5.5 respectively and[18] they are precipitated by a wide variety of substances. Urinary proteins are identical chemically and physically with serum proteins,[12] so that they display the same reactions as the latter.

Two important conditions must be present for complete precipitation of proteins. An adequate amount of salt must be present. Very dilute urines or those of low specific gravity require additional salt. This is best accomplished by the use of Sorensen's acetate buffer. Proteins have characteristic isoelectric points. Without attempting to explain the intricacies of colloidal chemistry, the isoelectric point of a protein may be described as that point at which the electric charges are neutral and therefore permit complete precipitation. An isoelectric point of a protein is just as individual a feature of that protein as a boiling point is of a liquid or melting point of a solid. Human serum albumin has an isoelectric point of 4.7, serum globulin 5.5. Hence, before a precipitant is added to a solution containing these proteins, it must be at or near pH 4.5 to accomplish complete precipitation.[19]

Proteins are precipitated in three forms: unaltered, as an albuminate, and as an insoluble salt. The first reaction takes place when large quantities of salts as: $(NH4)_2\ SO_4$, $Na_2\ SO_4$, NaCl, $Zn\ SO_4$, and so forth are added in solid form to the protein solution. Apparently, these salts so saturate the fluid or menstruum that it is no longer capable of retaining the protein in solution. It is precipitated by a process called "salting out," and employed in isolating the various protein fractions. Changes of dehydration and neutralization of protein molecules and molecular aggregates cause the precipitation. Alcohol acts not only through this dehydration phenomenon but produces intramolecular changes in the precipitation of protein through a change called coagulation.

A second form of protein precipitation occurs when certain metallic salts, e.g., $Hg\ Cl_2$, $Ag\ No_3$, $Cu\ SO_4$ $Pb\ (C_2H_3O_2)_2$ and so forth, are added.

Proteins are precipitated as insoluble salts on the addition of weak organic acids as certain of the alkaloidal reagents. Picric, phosphotungstic, phosphomolybdic, sulphosalicylic, tannic or chromic acids when added to a protein solution form insoluble salts. It is believe that there is a combination of the negative ions only on the acid sides of their isoelectric points. Even the sodium salts of these acids are incapable of producing this precipitation. However, the addition of a small amount of acetic acid readily causes the desired precipitate to form.

Preparation of Urine Before Precipitation.

From this discussion, it is obvious that protein precipitation is a complex mechanism. Certain conditions must be present before precipitation occurs, as proper acidity, salt concentration, time to form complete precipitate, and proper temperature. In hospital and clinical practice, urines in various conditions are submitted for analysis. For example, if freshly passed urine is not stored in a refrigerator or a preservative added, it soon becomes alkaline from the breakdown of its contained urea into ammonia. Such urine may show little or no precipitation on the addition of precipitating agents.

All urine specimens must be brought to a pH 4.5 before they are precipitated. This is best done by the addition of concentrated acetic

acid drop by drop, stirring vigorously between additions to expel liberated ammonia. Additions of concentrated acetic acid usually involve such small volumes of acid that dilution is not an important factor. Strongly alkaline urines or the presence of considerable buffer salts require large quantities of acid and the dilution must be calculated. Any of the commonly used indicators may be employed. Nitrazine paper worked admirably in our experience. By comparison with known color standards a pH of 4.5 or less is easily obtained. Low specific gravity urines have too low a salt contraction, so a small amount of Sorenson's acetate buffer must be added. We have not encountered this difficulty.

Urine must be at a given temperature to secure uniform precipitation. All our experiments have been done at room temperature. Urine specimens that have been stored in the refrigerator must be brought to room temperature before testing. All flocculation tests were allowed to stand ten or more minutes before they were read. From twenty to twenty-four hours were needed before the Esbach readings were recorded.

Difficulties Encountered in Previous Methods.

During the comparative study of these four methods over the last three years, Doctor H. F. Hailman and I have encountered certain difficulties. Not infrequently after twenty-four hours the precipitate of the Esbach test failed to completely settle down in the tube to an accurate level. In other instances, a part or even all of the precipitate would float. Readings then became largely estimates or guesses at total volume of precipitate. Another drawback is the time element or the twenty-four hours to collect the patient's urine and then another twenty-four hours to read the values. This means the clinician is two days behind in obtaining the results. Also, it is impossible to determine protein values of more than fifteen grams per liter. We have tried to dilute urine so that its precipitated protein could be read on the Esbach scale but found great variations with different degrees of dilution. All dilutions were made with 0.2% NaCl in the hope of not disturbing the hydration aggregates of the protein molecule. Various other salt concentrations were tried, but we were unsuccessful in getting proportionate readings with proportionate dilutions.

The Shevky-Stafford method was a disappointment. It was very difficult to consistently get duplicate readings with the same urine. Too frequently the bottom of the centrifuge tube was packed down by a firm precipitate over which a light film layer of variable density lay. Since two radically different agents are used in this test, i.e., phosphotungstic acid and alcohol, two different precipitates form and the firmness of packing of them is not uniform. Also, the correlation between this method and the Van Slyke was only fair.

Several objections to the Kingsbury-Clark method arose. Standards ranged from 10 to 100 mgm. of protein per 100 cc. of urine. Urines containing these quantities of protein showed a good correlation to the Van Slyke results. When larger amounts of protein were present, dilution was necessary. In our experience, protein solutions did not always give proportionate readings when accurately diluted with salt solution. A good comparator box is essential. The metallic box designed by Kingsbury-Clark works well. It is fitted with eight automobile lamp bulbs wired in series. These bulbs are sixteen volts, a size no longer used in current automobile industry and fairly difficult and expensive to replace. We have built a small comparator box with a fluorescent bulb that both works very well and obviates the previous objections.

Determination of protein by measurement of its nitrogen content by the Van Slyke manometric method has long been considered standard. The total nitrogen content of urine is first determined and then the non-protein nitrogen is ascertained. Protein nitrogen is thus total nitrogen minus non-protein nitrogen. A word of caution should be interjected at this point. In those urines that have a low protein content, i.e., where the non-protein nitrogen comprises nearly all of the total nitrogen, the likelihood of error is great. For example, if the total nitrogen of a urine sample is 2620 mgm. and the non-protein nitrogen is 2010 mgm., then the protein nitrogen is 2620-2010 or 610 mgm. Consequently, a 3% error in either the total nitrogen or non-protein nitrogen would result in a 10% error of protein nitrogen.

New Method.

An appraisal of the currently employed methods of protein determinations disclosed some limitations and objections to each of them.

The Esbach method seemed to give the most reliable results. However, there were two disadvantages frequently encountered, the uncertainty of a well packed precipitate at the bottom of the tube and difficulties in determining high protein concentrations.

With these objectional features in mind, a new method[20] was devised. It was rapid, accurate, and overcame previous difficulties. Briefly, it is as follows: Urine specimens to be tested must be at room temperature and acid to pH 4.5. If originally alkaline, concentrated acetic acid is carefully added drop by drop, stirring to liberate the ammonia. Exactly 4 cc. of urine and 4 cc. of Esbach reagent are pipetted into a special 10 cc. centrifuge tube, whose last cubic centimeter is drawn out to a fine tip. The mixture is stoppered and inverted three times to insure complete mixing. It is then allowed to stand for five minutes to facilitate complete precipitation of protein. After that, it is centrifuged at 1800 revolutions per minute for exactly fifteen minutes. The amount of precipitate is read to the nearest 0.01 cc. One cubic centimeter of precipitate by this method represents 1963 mgm. of protein per 100 cc. of urine. By comparison with results obtained by the Van Slyke manometric method, the new procedure was an accurate and reliable method. Duplicate checks varied not more than 0.02 cc.

Few additional instructions are needed. Urine that contains large amounts of protein so that the precipitate in the bottom of the tube is in excess of 1 cc. is less accurately determined by this method. The test tube begins to widen above the 1 cc. mark and the 0.1 cc. marks are very close together making readings of 0.01 cc. impossible. As a fairly large factor is employed, these differences are exaggerated. The method is quite unreliable in testing undiluted urines containing more than 20.00 Grams of protein per liter. When such urines are diluted with 0.2% NaCl and tested satisfactory results are obtained. On the other hand, urines that contain less than 100 mgm. of protein per 100 cc. are not satisfactorily tested by this method. The Kingsbury-Clark method is thoroughly reliable for these mild proteinurias.

Conclusions.

Quantitative estimations of the amount of protein in urine are of help in judging the treatment and prognosis of certain cases of

Bright's disease. While many methods have been suggested, there are limitations or objections to all of them. The most reliable tests in our hands have been the Kingsbury-Clark method for urines that contain less than one Gm. of protein per liter, and an improved centrifuge Esbach method for larger amounts. Since the flocculation and precipitation of protein is a very complex reaction, urine must be under a definite uniform condition before the precipitant is added. This includes the proper temperature, pH and salt concentration. When these conditions are satisfied the above mentioned tests correlate very well with results obtained on the same urines by the gasometric Van Slyke method. The Kingsbury-Clark and the improved Esbach tests are simple accurate quantitative tests for proteinuria.

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THE SULPHONAMIDES AND THE ZIEHL-NEELSEN STAIN

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Recently, we received daily specimens of sputum from a patient recovering from an attack of pneumonia. The Doctor requested an examination for acid-fast bacilli. We made smears and stained them by Ziehl-Neelsen method and carefully examined each slide. With moderate frequency, we encountered what appeared to be acid-fast rods. They certainly kept the carbol-fuchsin stain, but they looked a little bit too long and a trifle too slender to be catalogued as the tubercle bacillus. Puzzled, we called the Doctor and asked him to take a look at the slides. He agreed that they were acid-fast rods and that they looked "rather suspicious." He ordered a more careful examination of the sputum specimens from this patient. She was still running a temperature and was coughing and expectorating quite a bit.

Each day we discovered the same long, slender, acid-fast rods. Sometimes they were curved, and sometimes they were short and straight. I noticed some in bristling clumps. Then something clicked in my mind. Where had I seen wisps of thread-like rods or organisms or crystals, perhaps, bunching together like that? I recalled centrifuging urine specimens over and over preparatory to looking for sulphapyridine crystals. I remembered that upon examining the centrifuged urinary sediment, I came across bristling, thread-like crystals, some very thin, others shredded, but all looking more or less like the "acid-fast rods" I had now before me on the sputum smears. I remembered also that these crystals were those I had been looking for.

I asked the Doctor if he had ordered sulphapyridine, or sulphathiazol for his patient. He said that earlier in the course of her sickness, he had ordered sulphathiazol, and then discontinued it.

After a lapse of a day or two, he ordered sulphapyridine. Then I asked him if either of these drugs would take the acid-fast stain. He confessed that, to his knowledge, the question had never come up.

I procured tablets of sulphanilimide, sulphathiazol, and sulphapyridine from our pharmacy. I dissolved each tablet in a separate beaker of distilled water. Then I made smears of each solution and placed the slides in the incubator until they were well dried out. The drugs had crystallized out on the slides. I then stained them with the Ziehl-Neelsen stain and examined them under oil immersion. Each one of those crystallized drugs failed to decolorize with acid-alcohol and what is more, they looked identically like the "rods" we found in the sputum under examination.

Having made this discovery, I thought it might be of interest to fellow-technologists who may have run into "acid-fast rods" in the sputum of non-tubercular patients. A technician's report of the sputum examination could doom a patient to a sanatorium for months. The stained crystals of the above-mentioned drugs are very misleading to the unwary and the fact that they do take the stain must be kept in mind in all future sputum examinations.

DIOXAN METHOD FOR TISSUE PREPARATION*

By DOROTHY L. SHAW, B.S., M.T.

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An outstanding contribution to the tissue technician has been the work of Graupner and Weisberger published in 1931 on Dioxan as a dehydrating agent. Following this, numerous others have contributed to the fund of information concerning dioxan, notably Thelma T. Baird, H. W. Mossman, D. A. Johansen, Paul Ralph and others. They have compared dioxan with the other dehydrating agents in common use and from their work we are given these conclusions. First, that dioxan, regardless of the fixative does not render tissue hard or brittle during the dehydrating process; second, it shortens the time of the paraffin method and yet offers a wider time variation; and third and most important, shrinkage and hardening of tissue, generally attributed to hot paraffin is seen to be directly related to the method of dehydration and preparation of tissue for the immersion in the melted paraffin, rather than the effect of hot paraffin.

The fluids commonly employed for dehydration are powerful desiccators, and all living organisms contain water and possess the capacity for absorbing water. Johansen made the observation that the most satisfactory cases of infiltration were when the free, and not the combined water, was removed from the tissue, that is, when dehydration was produced without desiccation. In other words, good infiltration occurs when the water is replaced, but the water absorbing capacity of the tissue is not destroyed. Dioxan fills this requirement perfectly.

Dioxan is 1:4 diethylene dioxide. It is a colorless liquid having a pleasant ethereal odor. It is completely miscible with water, ethyl alcohol, clearing oils and melted paraffin; it dissolves balsam, gum dammar, clarite, and isobutyl methacrylate polymer. Dioxan is in-

* *Presented before the Illinois Society of Clinical Laboratory Technicians.*

flammable, but has about the same flash point as 95% alcohol. The boiling point of ethyl alcohol is 78.5°C and that of dioxan 100.8°C. The vapor pressure of ethyl alcohol at 20°C is 43.9 mg. Hg. and that of dioxan 29.0 mg. Hg. This relatively low vapor pressure combined with moderate boiling point contribute to its ease of removal from the paraffin bath.

Dioxan vapor in concentration of 1:1000 parts of air is poisonous and prolonged exposure to such concentrations results in pathological damage to liver and kidneys. Such industrial concentrations are not encountered in the laboratory, but it is well to remember that dioxan is toxic and avoid inhaling the fumes.

The cost of dioxan per gallon is approximately five times the cost of ethyl alcohol. However dioxan is more economical. It can be used over and over and alcohol loses its value as a dehydrating agent after the first usage.

Thorough fixation is a prerequisite to all good tissue work, regardless of the method. With the dioxan technique it is imperative. Alcohol and xylol produce enough hardening of the tissue to counteract any slight inadequacies of fixation, but with dioxan a soft area remains soft throughout the entire process of dehydration and infiltration. This soft area will be difficult, if not impossible to cut. The paraffin mattrix will not be strong enough to hold it in place, with consequent loss of this area either at the time of cutting or when the section is floated on the water.

The usual fixing reagents are satisfactory and 24-48 hours is adequate time for all sections. In any step in the preparation of a good slide, thickness always alters time, from the fixation to the drying of the mounted section on the slide, prior to staining. So when I mention time, I am speaking of average thicknesses in all instances. Our routine fixation is 10% formalin for 24 hours and the pieces of tissue are not over ½ inch in thickness. After fixation, it is not necessary to wash the formalin out of the tissue as formalin pigment is soluble in dioxan. But a few minutes washing makes the handling of specimens more pleasant for the pathologist who trims the piece you are to section.

The section now goes into the first dioxan. It is labeled by means

of a wooden block (the block on which it will subsequently be mounted), attached to the dioxan jar with a rubber band. The dioxan jar is a preparation dish, tall form, outside diameter 62 mm., outside height 93 mm. with molded glass cover. The tissue is supported in the solution in a perforated porcelain cup. The reason for, or the advantage of, using the porcelain cup is that the tissue is kept free from any particles that might have fallen off sections previously carried through the solution, as any fragments drops to the bottom. The cup is available from Arthur H. Thomas, the number is 4381 and it costs $1.80.

There are three changes of dioxan. First dioxan 30 minutes, second dioxan one hour, and third dioxan for two hours. The tissue is transferred from the cup in the first dioxan jar to the second dioxan jar, or sometimes it may be more convenient to transfer the cup from one solution to the next.

The only pure or relatively pure dioxan solution is third dioxan. After a solution has been used as the final solution 5-10 times depending on number of pieces of tissue carried through it is moved down and used as second dioxan. After 5-10 times usage as second dioxan it will be moved down again and become the first dioxan solution. The number of times a solution is used is purely arbitrary and the time to discard it can better be told by the appearance. When as first dioxan it becomes discolored, cloudy and dirty, discard it. Some water in the solution is not undesirable. The specific gravity varies from 1.035 with undiluted dioxan to 1.042 in a solution containing 70% of water. At that stage the solution is theoretically of no more value and should be discarded. In practice, I have not determined the amount of water in discarded solutions, but from their appearance would estimate they were 50% water.

Some have recommended the use of calcium oxide to dehydrate the dioxan, but I found it unsatisfactory during the short time I tried the method. Since water in the first and second change of dioxan is not objectionable, I have not found it worth while to try to overcome the difficulties I encountered using dioxan over calcium oxide.

After the three changes of dioxan there are three changes of

paraffin. The first paraffin bath for 15 min., the second for 45 min., and the last for two hours or longer. The time may be adjusted to suit your convenience. The periods designated are minimum except for very small pieces such as skin biopsies. The section may be left hours in any of the six changes, with no damage resulting. I generally leave sections in third dioxan for three or four hours and then overnight in third paraffin, embedding the first thing the next morning. For the past three years we have been using Fisher's Tissuemat Melting point 60-62°C. I like Tissuemat instead of ordinary paraffin because on those sections which used to be hard to cut such as breast tumors, uterus and those pieces which are harder in consistency than average, they are as easy to cut as almost any other type of tissue. Tissuemat seems to furnish more support. I like the higher melting point for the same reason and in the summer time the only time I have to use ice to cool the block is when I need more sections than I can cut in a couple of minutes.

These paraffin baths are used over and over again just as the dioxan is, only much more so. About once a month I convert third paraffin into second; second paraffin to first; discard the first and melt some fresh for third.

When it comes to embedding I prefer watch glasses to paper boxes. Paper boxes are convenient for some types of tissue, such as prostate resections. But watch glasses save a lot of time. First I mark the number on the inside of the glass while it is inverted. Looking at the inverted bottom as you write the number on it with the wax pencil. It appears exactly as it will on the cake of paraffin when it floats free from the glass. Put a film of moisture on the inside of the glass by breathing on it, this keeps the paraffin from sticking. Fill to the desired depth with paraffin and place the sections in this in the desired position. Varying the size of the watch glass according to the number of pieces of tissue you have to embed under that number, you will find you can embed from 1 to 20 pieces. Do this embedding fast enough so that there is no film over the paraffin when you have finished. Float the watch glass in a dish of cold water until the film which now forms is dense enough to protect the paraffin when it is submerged. When the mass has cooled sufficiently it will automatically float to the surface free from the watch glass

and on the bottom will be the number you wrote on the watch glass. In a few minutes it will be ready to block. After removing from the water, dry with a towel and then hold up to light to find the proper places to cut so that each section will have a quarter of an inch of paraffin around it. Make these cuts or scratches from one edge to the opposite in a nearly straight line. A straight line enables you to break the paraffin into two pieces without breaking any of the sections. Trim off excess paraffin and attach to wood block with aid of a hot spatula. If you have 10 or 20 pieces to block at a time be sure that the blocks are put in the refrigerator within 3 or 4 minutes after blocking instead of leaving at room temperature until you have finished. Paraffin has to be cooled quickly or it will crystallize and crystallization handicaps cutting. After the blocks have been refrigerated for 15-30 minutes they are ready for cutting.

Dioxan is also used in the staining process in place of alcohol. Dioxan dissolves cold paraffin slowly, so we use 2 changes of xylol to remove the paraffin followed by 2 changes of dioxan. After staining we dehydrate with 2 changes of dioxan, clear with 2 changes of xylol and mount in 50% Clarite in xylol.

I use a three-day procedure for this process. First 24 hours for fixation, second 24 hours for dehydration and infiltration and on the third day, block, cut, and stain. Individual sections can be carried through in much less time than this, but when there is no rush, this three-day schedule, having the slides ready for diagnoses the morning of the fourth day saves a lot of my time, and every technician is interested in saving time where it can be saved.

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A TECHNIC FOR CROSS MATCHING WITH A LIMITED QUANTITY OF BLOOD

By JACK M. AARONS, M.T.

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In many pediatric cases where a transfusion is necessary, it is often a problem to obtain a sufficient quantity of blood to do a satisfactory cross matching by any of the ordinary methods where 6 to 8 c.c. are necessary. Due to this difficulty the author has devised a method by which most satisfactory typing and cross matching may be done on less than 2 c.c. of blood obtained from a puncture in the finger, toe, or ear. This method was checked on numerous cross matchings done in the Laboratory Department, William Beaumont General Hospital, El Paso, Texas; Serology Laboratory, Parkland Hospital, Dallas, Texas; and more recently on those done in the Laboratory Department, Station Hospital, Camp Hulen, Texas. It was found that compatibility or incompatibility checked 100% with both the Culbertson and Rotcliffe[1] test tube technic and a slight modification of the technic of Wiener[2].

The method is simple and consists of using plasma obtained from citrated blood rather than serum. The citrate does not interfere in any way with the test.

The patient's group may be determined by obtaining a small quantity of blood from a finger, toe, or ear puncture on a wood applicator and mixing with A and B serum on a glass slide. However, this procedure has been shortened by doing a typing on the cells obtained for the cross match.

Method No. 1

From toe, finger, or ear of patient collect about 10-12 drops 1-2 c.c.) blood into a small tube (about ⅜ x 3 in.) containing 0.5 c.c. of 1% sodium citrate in 0.85% sodium chloride and mix well.

Centrifuge at a moderately high rate of speed for 5 minutes to throw down the cells.

Draw off plasma from cells with a medicine dropper or capillary pipette and deposit into another similar small tube.

Determine patient's group using A and B serum and adding cells with a wood applicator and rotating slide gently until agglutination occurs. Agglutination will usually occur in 3 to 5 minutes if typing serum is of sufficient high titer.

Technic for Cross Matching

Place 1-2 drops of patient's plasma and 1-2 drops of donor's plasma on a paraffin or wax ringed slide, labeling them A and B respectively.

With a wood applicator add a small quantity of donor's cells and patient's cells to A and B respectively.

Rotate gently for 3-4 minutes and observe macroscopically and microscopically for agglutination. Agglutination will usually occur before 5 minutes. However it is best to wait 20 minutes or longer before reporting cross matching acceptable.

Method No. II

Obtain blood, centrifuge and draw off plasma as in Method No. I. Dilute cells with saline until a nice suspension (judged by the eye) is obtained and using this suspension determine patient's group by adding 2 drops to 2 drops A and B serum in separate small test tubes, centrifuging at a high rate of speed for 3 minutes, and observing macroscopically and microscopically for agglutination.

Technic for Cross Matching

Place 2 drops patient's plasma and 2 drops donor's cells along with 2 drops saline in a small test tube. Likewise place donor's plasma and patient's cells and saline in a second test tube, labeling them A and B respectively. Shake well to mix.

Incubate at 37.5 degrees Centrigrade in water bath for 5 minutes. If hemolysis is going to occur, it will usually do so at this time. If

no hemolysis is noted, centrifuge for 3 minutes at a high rate of speed.

Remove from centrifuge, note evidence of hemolysis, shake tubes to resuspend cells, observing clumping if present.

Pour fluid on slide and examine microscopically for agglutination.

Summary and Conclusions

1. A choice of technics for cross matching with a limited quantity of blood has been presented.

2. Of the two methods, preference is given Method No. II, because of the fact that it is more sensitive, the agglutination is accentuated, hemolysis if present may be more readily detected, and it is far less time consuming.

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2. Wiener, A. S.: Blood Group and Blood Transfusions. 2nd Edition, 306 pages, C. C. Thomas, Springfield, Illinois, 1939.

ABSTRACTS

JAUNDICE IN INFANTS AND IN CHILDREN. The Icterus index as a method of determining the type of jaundice: M. Reimer & S. Weiner, Am. Jr. Dis. Child., Vol. 61, No. 4, April, '41, p. 752.

Icterus index is read in the usual way against potassium dichromate using physiological salt or water as a diluent. Proteins are precipitated with acetone and any hemoglobin present is removed. The supernatant fluid is also read against potassium dichromate. Normal for the acetone method is 5 units with 8 units clinically evident. The ratio of the index obtained by the water method to that by the acetone method is 1:1 in pure hemolytic jaundice. Icterus neonatorum was thus found to be in this group.

LABORATORY INFECTIONS DUE TO BRUCELLA: K. F. Meyer & B. Eddie, Jr. Inf. Dis., Vol. 68, No. 1, Jan., '41, p. 24.

Published records of 74 cases of brucellosis occurring among laboratory workers and laboratory animal caretakers in the United States are summarized. All three types of Brucella were demonstrated. Attempts to immunize with antigen injections with dead vaccines failed. Handling of Brucella material and inhalation of dust containing the organisms is dangerous to laboratory personnel.

A BACTERIAL ASSAY METHOD FOR NICOTINAMIDE AND RELATED SUBSTANCES IN BLOOD, URINE AND SPINAL FLUID: H. Isbell, J. G. Wooley, R. E. Butler and W. H. Sebrell, Jr. Bio. Chem., Vol. 139, No. 2, June, '41, p. 499.

The growth of Shigella paradysenteriæ in an ammonium lactate medium has been reported to be, within limits, proportional to the amount of nicotinamide and related substances present in the medium. A series of standard tubes containing known amounts of nicotinamide are set up each time with the assay tubes and all are autoclaved. They are then inoculated with equal amounts of a suspension of the organisms and incubated. Turbidity is read in the photometer. The standard curve is plotted and the concentration of

the assay material taken from it.

All materials for the assay must be freshly prepared and the standard curve plotted each time. Nicotinic acid and related substances cannot be distinguished and the results are expressed as "microgram equivalents to nicotinamide."

A CYTOPHAGIC REACTION EMPLOYED IN THE DIAGNOSIS OF BRUCELLA INFECTION: M. Jerslid, Jr. Inf. Dis., Vol. 68, No. 1, Jan., '41, p. 16.

A method is presented using inactivated patient's serum, the usual suspension of organisms and citrated blood from a known negative donor. This eliminates the necessity of obtaining patient's blood within 6 hrs. of the test and, as the phagocytes are obtained just prior to the test, they are more active. Inactivation eliminates some of the non-specific reactions.

THE FROG TEST (XENOPUS LAEVIS) AS A RAPID DIAGNOSTIC TEST FOR EARLY PREGNANCY: A. I. Weisman & C. W. Coates, Endocrinology, Vol. 28, No. 1, Jan., '41, p. 141.

The previous results of this test are summarized. Injection was made in the dorsal lymph space and ovulation occurred within 6-18 hrs. when 100 R.U. or more of anterior pituitary-like hormone was used. Normal urines are not likely to contain this much. Accuracy up to 99% was reported in comparison with the Aschheim-Zondek test and it is said by some to be more accurate than the Friedman.

THE ELECTRON MICROSCOPE: A NEW TOOL FOR BACTERIOLOGICAL RESEARCH: L. Marton, Jr. Bact., Vol. 41, No. 3, March, '41, p. 397.

Detailed description with diagrams of the mechanism of this new type of microscope using a beam of electrons from a tube similar to an X-ray tube, in place of light. The image is viewed on a fluorescent screen or a film is exposed. Since electrons are readily diverted by even a few molecules, the procedure must be carried out in vacuum and preparations must be mounted on extremely thin collodion films instead of glass. At present preparations are not stained. Photographs of virus particles as well as ordinary organisms are given in magnifications of 7,000 to 20,000 diameters.

THE EFFECT OF SYPHILIS AND ITS TREATMENT ON THE INCIDENCE OF TOXEMIA OF PREGNANCY: C. H. Peckham, Am. Jr. Syph., Gon., & Ve. Dis., Vol. 25, No. 3, May, '41, p. 280.

Treatment of syphilitics was begun either before or during pregnancy as the condition became known but these women showed no higher incidence of the toxemias of pregnancy than the non-syphilitic group.

THE MAINTENANCE OF AEROBIC, MICRO-AEROPHILIC AND ANAEROBIC CONDITIONS IN A PETRI DISH: A. Cantor, Jr. Bact., Vol. 41, No. 2, Feb., '41, p. 155.

All three conditions were maintained by the use of watch glasses or small discs on glass supports and deep agar in a single poured plate. Sodium bicarbonate was added to the media as a source of carbon dioxide. When this was autoclaved in tightly closed screw-capped bottles, the carbon dioxide was retained whereas in cotton-plugged bottles it was lost.

EFFECT OF EDEMA AND INTEGUMENTARY INFILTRATIONS ON BASAL METABOLISM, ELECTROCARDIOGRAM AND BLOOD CHOLESTEROL: E. Moschcowitz, Arch. Int. Med., Vol. 67, No. 4, April, '41, p. 828.

The author discusses theories advanced to explain the low BMR characteristic in edema with the conclusion that the edema acts as a suit of clothes preventing transfer of heat. Two case reports of indurative scleroderma and one of prolonged cardiac failure are given. Blood cholesterol content is not directly related to the BMR but is influenced by thyroid.

CULTURE OF TUBERCLE BACILLI, An Improved Technique: W. Steenken, Jr. & M. M. Smith, Amer. Rev. Tuber., Vol. 43, No. 2, Feb., '41, p. 309.

Description of a technique in which malachite green is added to the digested sediment of sputum, urine, pleural or gastric fluid, to reduce the contaminants. The authors obtained more positive cultures in shorter time using sediment treated this way on the modified culture media they suggested in a previous publication than on the Hohn's medium containing malachite-green. A pH of 6.2 was found preferable to the usual 7.2.

BACTERIAL MORPHOLOGY AS SHOWN BY THE ELECTRON MICROSCOPE. Structural Differentiation Within the Streptococcal Cell: S. Mudd & D. B. Lackman, Jr. Bact., Vol. 41, No. 3, March, '41, p. 415.

A group of electron micrographs of *B*-hemolytic streptococci in their three phases. These plates indicate that streptococcal cells have a rigid outer membrane which is continuous and accounts for their occurring in chains.

THE PHOTOELECTRIC DETERMINATION OF INDOLE IN BACTERIAL CULTURES: A. R. Stanley & R. S. Spray, Jr. Bact., Vol. 41, No. 2, Feb., '41, p. 251.

Indole is extracted from a measured amount of culture media by means of iso-amyl alcohol. An adaptation of Ehrlich's reagent is used to develop the color. Directions are given for the plotting of the indole curve.

STUDIES ON THE MECHANISM OF RECOVERY IN PNEUMOCOCCAL PNEUMONIA. I. The Action of Type Specific Antibody Upon the Pulmonary Lesion of Experimental Pneumonia: W. B. Wood, Jr. Exp. Med., Vol. 73, No. 2, Feb., '41, p. 201.

This work supports Loeschcke's observation that the pneumonic lesion spreads to adjacent alveoli by the spread of edema fluid containing the organisms. Microscopic observations showed an outer zone with many Pneumococci floating in edema fluid, a zone of leucocytes and organisms with some phagocytosis and an inner zone where the alveoli contain many leucocytes but no organisms. The administration of antiserum caused agglutination and capsular swelling of the Pneumococci especially in the edema zone and increased phagocytosis.

DISCOVERY OF HUMAN HEARTWORM INFECTION IN NEW ORLEANS: E. C. Fause, E. P. Thomas and J. Jones, Jr. Paristology, Vol. 27, No. 2, April, '41, p. 115.

Description of a heartworm found in a New Orleans woman which had apparently no effect on the individual. The worm differed sufficiently from that common in dogs and from that reported in a boy of Rio de Janeiro to be considered a new species. The origin or alternate host is entirely unknown.

ADAPTATION OF THE FOLIN-MALMROS MICRO BLOOD SUGAR METHOD TO THE PHOTOELECTRIC COLORIMETER: S. M. Horvath and C. A. Knehr, Jr. Biol. Chem., Vol. 140, No. 3, Sept., '41, p. 869.

The Folin-Malmros method was adapted for the Evelyn photoelectric colorimeter for a range of 50-300 mg.%. Substitution of Duponal for gum ghatti and the use of a 20-minute boiling time gave more constant results.

CARBOHYDRATE STORAGE AND MOBILIZATION WITH CHANGES in the BLOOD pH: M. Guest & R. Rawson, Jr. Biol. Chem., Vol. 139, No. 2, June, '41, p. 535.

The rate of body pH was varied by intraperitoneal injections of various solutions. Blood was withdrawn from the heart and liver and muscle tissue was taken at the same time. Liver glycogen was found to vary directly with the blood pH up to 7.3 when no relationship obtained. Blood sugar varied inversely with the pH and muscle glycogen was unaffected by it.

THE GRAVIMETRIC DETERMINATION OF BLOOD SERUM PROTEINS: H. W. Robinson and C. G. Hogden: Jr. Biol. Chem., Vol. 140, No. 3, Sept., '41, p. 853.

Three and five cc. samples were treated with acetone to ppt. protein and to remove lipids. Separation was accomplished by centrifuging. Ppt. was heated in water bath 1 hr. and after washing with water, alcohol and ether was dried to constant weight at 105-110°. Values for this method were slightly lower than for the Kjeldahl. The greatest difference recorded was 0.29g. per 100 cc. serum.

THE BLOOD CELLS IN EARLY SYPHILIS: V. J. Wile, R. Isaacs, C. W. Knerler, Am. Jr. Syph., Gon., & Ven. Dis., Vol. 25, No. 2, Mar., '41, p. 133.

Study material was early active syphilis in human being and in rabbits. Mild anemia of the secondary type, monocytosis and eosinophilia were found, as previously reported by other investigators. New findings recorded were the frequent occurrence of plasma cells and a type of cell similar to that characteristic of infectious mononucleosis.

NEWS AND ANNOUNCEMENTS

DR. JOSEPH B. DeLEE AWARDED 1941 MISSISSIPPI VALLEY MEDICAL SOCIETY DISTINGUISHED SERVICE AWARD

Dr. Joseph B. DeLee of Chicago, Illinois, Professor Emeritus of Obstetrics and Gynecology, University of Chicago, was awarded the Mississippi Valley Medical Society Distinguished Service Award for 1941 at the annual banquet of the Society held at Cedar Rapids, Iowa, October 2. The award is given annually to an active member of the Society for "unusual and distinguished service to the medical profession." The citation of the annual awards committee of the Society in connection with Dr. DeLee's award was, "Joseph Bolivar DeLee, physician, humanitarian, foremost in obstetrics, a credit to himself, his associates, his profession and his country."

This is a most fitting tribute to a physician who has devoted his life to medical teaching at Northwestern University and the University of Chicago. Dr. DeLee has been the guiding light for many years in the great Chicago Lying-In-Hospital. He has been a voluminous contributor to the obstetric literature, which is evidenced by the fact that for many years he has been co-author of the "Year Book of Gynecology and Obstetrics." His well-known test, "Principles and Practices of Obstetrics," has had seven editions while his text, "Obstetrics for Nurses," is now in its twelfth edition and his "Notes on Obstetrics" has had two editions. In addition to this he has made fifteen sound motion pictures on various obstetric subjects. Dr. DeLee immediately recognized the educational work of the Mississippi Valley Medical Society and is one of its charter members.

MISSISSIPPI VALLEY MEDICAL EDITORS' ASSOCIATION ELECTS OFFICERS FOR 1942

The first annual meeting of the Mississippi Valley Medical Editors' Association was held in Cedar Rapids, Iowa, October 1, under the presidency of Dr. George B. Lake of Waukegan, Illinois, editor of *Clinical Medicine*. The program consisted of the following:

"Value of the Exchange of Ideas in Medical Writing for the Mississippi Valley" by Henry G. Langworthy, M.D., Dubuque, Iowa; "Science and Art in Medical Writing," by Paul E. Craig, M.D., Coffeyville, Kansas; "Publication Make-Up," by Dr. Florence Johnston, Cedar Rapids, Iowa. These papers were followed by an enthusiastic general discussion.

A constitution for the new organization was adopted containing an article stating "The purpose of this Society shall be to bring into one organization all physicians in the Mississippi Valley who are members of the American Medical Association, and who are interested in medical journalism or medical writing, and any laymen who are connected with the editorial or business staffs of medical periodicals, so that by interchange of views, they may secure such intelligent unity and harmony in every phase of their labor as will elevate and make effective the opinions of the medical profession in their respective communities."

The new organization will hold annual meetings at the time and place of the Mississippi Valley Medical Society meeting. The officers elected for 1942 were as follows:

President, Dr. Clyde P. Dyer of St. Louis, Mo., editor, St. Louis County Medical Society Bulletin; president-elect, Dr. James Dunn of Davenport, Ia., editor, Bulletin of the Iowa and Central Illinois District Medical Association; vice-president, Dr. Elliott F. Parker, Moline, Ill., editor, Medical-Dental News of the Quad Cities; secretary (reelected), Harold Swanberg, M.D., Quincy, Ill., editor, Mississippi Valley Medical Journal.

The following were elected to membership on the Executive Committee: Dr. George B. Lake, Waukegan, Ill.; Dr. Julius Jensen,

St. Louis, Mo.; Dr. Mathias J. Seifert, Chicago, Ill.; Dr. Henry G. Langworthy, Dubuque, Ia.; Dr. T. F. Hersch, Cedar Rapids, Ia.; Dr. Paul E. Craig, Coffeyville, Kans.

Efforts are being made to expand the association and all interested in medical journalism or writing are cordially invited to membership. Application blanks can be secured from the Secretary, Dr. Harold Swanberg, 209-224 W.C.U. Building, Quincy, Ill. The Association is also endeavoring to establish a Cooperative Advertising Bureau to serve the smaller publications of the Mississippi Valley area in a similar way as does the Cooperative Advertising Bureau of the American Medical Association serve the State Journals.

Chicago

The Chicago Society of Medical Technologists held a dinner meeting Thursday, September 18th, at 6:30 at the Army and Navy Club, 600 South Michigan. Guest speaker was Major Hugh Gilmore, M.C. of Fort Sheridan, speaking about the Medical Technologists in the Defense Program.

The October meeting was held the 24th. There was a tour of the new Abbott Memorial Laboratories of the Evanston Hospital and a movie, "Canoeing Through the Colon."

The 1941-42 officers are:

President—Augusta Sturmer, 826 E. 61st, Chicago.

Secretary—Lois Mansnerus, c/o Dr. Richter, 6 No. Michigan.

Treasurer—Elizabeth O'Connor, 313 N. Park Ave., Highland Park, Ill.

Illinois

The annual fall meeting of the Illinois Society of Clinical Laboratory Technicians was held October 24-25, 1941, at the University of Illinois, Urbana-Champaign.

The following program was presented:

Friday Afternoon, October 24—

Registration—Burnham City Hospital Laboratory, 310 East Springfield Avenue, Champaign, Illinois.

Friday Evening, October 24—

Room 112, Chemistry Annex, University of Illinois, Margaret Church, Presiding.

7:30—Greetings from President.

7:45—"Researches on Vitamin E Deficiency," F. B. Adamstone, Associate Professor of Zoology, University of Illinois.

8:30—"Gastroscopy—A Diagnostic Aid," C. H. Drenckhahn, M.D., Carle Hospital Clinic, Urbana, Illinois.

Saturday Morning, October 25—

Room 112, Chemistry Annex, Margaret Church, Presiding

9:00—"Microbiological Methods of Vitamin Assay," John C. Garey, Instructor in Bacteriology, University of Illinois.

9:40—"Rocky Mountain Spotted Fever and Method of Preparing Vaccine," W. Wayne Lockwood, M.D., Carle Hospital Clinic, Urbana, Illinois.

10:45—"The Training of Medical Technologists," Lall G. Montgomery, M.D., Chairman, Board of Registry, Muncie, Indiana.

11:30—Business Meeting with Election of Officers.

12:00—Adjournment.

Room 112, Chemistry Annex, Margaret Church, Presiding.

1:15—"The Action of Protamine Zinc Insulin," J. E. Sexton, M.D., Christie Clinic, Champaign, Illinois.

2:00—"Uses of Radio-Active Materials in Biology and Medicine," P. Gerald Kruger, Professor of Physics, University of Illinois.

3:00—"Some Recent Advances in Blood Determinations," Julius Johnson, Research Assistant in Chemistry, University of Illinois.

4:00—Adjournment.

The following excerpt is from a news letter to members of the Illinois Society of Clinical Laboratory Technicians:

The Society is now officially affiliated with the American Society of Medical Technologists and the affiliation charter has been received. The A.S.M.T. is announcing, through its publication, an offer made by the American Society of Clinical Pathologists of an

award of fifty dollars to be given to the technologist who presents the best paper at the next A.S.M.T. meeting.

At the May meeting of the Illinois Society of Clinical Laboratory Technicians eighteen new members were accepted. This brings our total membership to 160. If you know of any prospective members write to Jessie K. Lex, or to the chairman of the membership committee, Elizabeth O'Connor, 313 Park Avenue, Highland Park, Ill., for application blanks.

We were sorry to lose from our rolls this past year Genevieve Stout of Oak Park, who accepted a position at Fort Custer, Michigan, and Norma Wonstrom of Peoria, who went to a new position in Florida. Norma Wonstrom was our vice-president for the current year. Her term of office has been filled out by Dolores Lammers, of the Illinois Department of Public Health, Chicago.

If you have any papers you would like to present at any future meetings the secretary would be pleased to be advised.

Please notify the secretary, Miss Jessie K. Lex, 927 Alliance Life Bldg., Peoria, of any change of address and also of any change in your status in the Society by virtue of having become registered with the Registry of Medical Technologists.

Minnesota

The slate of officers for the Minnesota Society of Medical Technologists is as follows:

President—Frances Anderson, Eitel Hospital, Minneapolis.

Pres.-Elect—Martha Strolberg, 601 So. Upton Ave., Minneapolis.

Past President—Agnes Hilden, St. Luke's Hosp., St. Paul.

Vice-President—Mary Conroy, Minneapolis General, Minneapolis.

Secretary—Esther Wilbrecht, New Ulm, Minn.

Treasurer—Valerie Olsen, 4023 Second Ave., S., Minneapolis.

Directors—Olga Nelson (1 yr.), Midway Hosp., St. Paul; Sr. Edeltrude, St. Joseph's Hosp., St. Paul; Marion McGee, Northern Pacific Hosp., St. Paul.

The official paper of the state society is *Minnesota Medical Technologist*. The cost of publication is in part through advertisers and from the reports, the advertisers are pleased with the response shown from last year's advertising.

Beginning with 1942 voting at the state society will be carried on by mail, a ballot to be sent to every paid member with the April issue of the Minnesota paper. The ballots are to be returned, properly used not later than the business session of the annual meeting of the society. This may be done either by mail or in person.

The University of Minnesota gave a course for Medical Technologists at the Center for Continuation Study on the Main campus, October 20, 21, 22, 1941. Enrollment was limited to members of the Registry of Medical Technologists of the American Society of Clinical Pathologists and non-members with equivalent training and experience.

This course was the second for medical technologists in the history of the center.

Each prospective student was circularized, a card and application blank being mailed him. The card bears a ruling in which the student may indicate what problems he wishes presented for solution at the course. There is space for three replies and a notation as to plans for attendance: "I will attend," or "I am uncertain." No signature is required. From the replies on the cards the program was built. In the past eminent pathologists and skilled technologists were professors in the course at Minnesota and the enrollment was not limited to the state but students were in attendance from beyond its boundaries.

Medical Technology is privileged to have the hospitality of the Minnesota Center for Continuation Study thrown open to it. The program of courses at the Center for Continuation Study during the past five years has included the following:

1. Practical instruction for professional groups.
2. Quiet, pleasant, well-furnished rooms for the convenience of registrants.
3. Faculty appointments based on the particular requirements of each program.
4. Excellent dining room service; tea in the lounge at four o'clock.
5. Schedules arranged for efficient adult learning.
6. Plenty of opportunity to ask questions.
7. University-chartered transportation to and from all off-campus demonstrations.

Wisconsin

The annual meeting of the Wisconsin Association of Medical Technologists was held on Saturday and Sunday, October 11 and 12, at the Pfister Hotel and the Milwaukee County Hospital.

The following speakers presented the following papers on Saturday:

Dr. Philip Voigt, Milwaukee: "Preparation and Uses of Human Serum and Plasma."

Dr. Jos. Boch, Milwaukee: "Biochemistry of the Liver."

Dr. Norbert Enzer, Milwaukee: "Present Day Opinion Concerning Jaundice."

On Sunday the program consisted of a tour of the Milwaukee County Hospital buildings.

The following officers were elected for 1941-42:

President—Alice Thorngate, Madison.

President-Elect—Sr. M. Corona Rohlik, LaCrosse.

Secretary—Elizabeth Kullmann, Milwaukee.

Treasurer—Grace Ballard, Milwaukee.

REGISTRY OF MEDICAL TECHNOLOGISTS
of the
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

The Registry takes pleasure in announcing that a new manufacturer is now preparing the jewelry that is distributed to registered Medical Technologists. No change has been made in the design of the jewelry, but the workmanship has been improved. The following items are available:

Solid gold pin - -	$2.50	Solid gold ring -	$6.50
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Sterling silver pin -	$2.00	Solid gold key -	$6.75

Orders should be sent to the Registrar,

MISS CARLITA R. SWENSON
Ball Memorial Hospital
Muncie, Indiana

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